Comparative Genomics to Unravel the Role of Oral Microbiome and Host Immunogenetic Variations in Periodontal Disease to Predict Virulence Factors and Potential Therapeutic Targets

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1. Abstract

Introduction: Periodontitis is a chronic inflammatory disease driven by dysbiotic plaque biofilms and a dysregulated host immune response, leading to the destruction of tooth-supporting structures and tooth loss. The disease is primarily mediated by key periodontal pathogens, while the overall oral microbiota plays a crucial role in maintaining health or promoting disease when disrupted. Host immunogenetic variation, especially polymorphism in Human Leukocyte Antigen (HLA) genes influence immune responses, thereby contributing to individual susceptibility to periodontal disease. However, the precise interplay between oral microbial diversity and host HLA variability remains inadequately understood.

Objectives: Comparative genomic analysis of human oral microbial genomes to identify potential diagnostic and therapeutic markers. To reveal the Immunogenetic variations associated with periodontitis by identifying potential HLA variants. To study the salivary microbiome diversity, HLA genetic diversity and to correlate with differential susceptibility to periodontal disease.

Methods: This study is an integrative approach combining comparative genomics, functional analysis, and immunogenetic profiling. A total of 192 oral bacterial genomes were analyzed to assess genetic diversity, phylogenetic relationships, and their functional diversity to identify key virulence factors and metabolic adaptation associated genes. Analysis of host immunogenetic variations were focused on identifying significant HLA alleles associated with health and disease. Additionally, integrated microbiome-HLA analysis was conducted to evaluate the influence of host immunogenetic variation on oral microbial composition and diversity, and their association with disease susceptibility.

Results: Comparative genomic analysis of 192 oral bacterial genomes revealed the identification of novel species as well as the taxonomic reclassification of existing oral bacterial species. Functional diversity analyses identified key virulence factors, including *dksA* and *clpB*, along with metabolic adaptation associated genes, highlighting their potential as diagnostic and therapeutic targets. Analysis of immunogenetic variation demonstrated significant associations with specific HLA alleles, including HLA-B15:01, HLA-DR7, HLA-B7, and HLA-A3 with respect to periodontal disease susceptibility. Further, integrated microbiota-HLA analysis revealed that host immunogenetic variation is significantly associated with oral microbial diversity. Notably, HLA-B15:01 emerged as a key immunogenetic mediator that may modulate oral microbiota composition by limiting the colonization of *Porphyromonas gingivalis*, maintaining microbial balance, and thereby conferring protection against periodontitis.

Conclusion: This study demonstrates the critical role of oral microbiome-HLA interactions in periodontal disease susceptibility. The findings provide a foundation for future research aimed at developing predictive biomarkers and personalized therapeutic strategies for improved disease management.

Keywords: Periodontitis, Human Leukocyte Antigen (HLA), oral microbiota, disease susceptibility and molecular markers.

2. Introduction

Periodontitis is a chronic, multifactorial inflammatory disease caused by dysbiotic plaque biofilms and dysregulated host immune response, leading to progressive destruction of the tooth-supporting structures [1]. Untreated periodontitis can lead to tooth loss, and has been linked to various disease conditions including cardiovascular diseases, diabetes, adverse pregnancy outcomes, etc. [2-4]. Key pathogens such as *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* (Red complex organisms) are pivotal in the progression of periodontitis.

Human Leukocyte Antigens (HLA) are a group of proteins found on the surface of nucleated cells that play a crucial role in the immune system. HLA alleles play a role in determining an individual's susceptibility to various diseases [5]. HLA genes are highly polymorphic, and may enhance or diminish the immune response against particular pathogens. The immune regulatory capacity of HLA extends to how host cells interact with bacteria at a molecular level [6].

The oral microbiota, the complex ecosystem of microbes inhabiting the human oral cavity, harbors several thousands of bacterial types. The oral cavity has the second largest and diverse microbiota after the gut. It primarily colonizes surfaces such as teeth, gums, tongue, cheeks, and the throat. Emerging evidence suggests that oral microbial composition and diversity is associated with susceptibility to periodontal diseases and other systemic inflammatory conditions [7].

Despite growing recognition of the oral microbiome-host immune interplay in periodontal disease, the precise mechanisms linking microbial diversity with host immunogenetic variability remain inadequately understood. In this context, the present study focuses on comparative genomics approaches to comprehensively investigate the genetic architecture of key oral microbial communities along with host HLA variations to elucidate their combined role in periodontal disease pathogenesis. The novelty of this study lies in bridging oral microbial diversity with host immunogenetic variation to uncover potential biomarkers, elucidate pathogenic mechanisms, and identify therapeutic targets, thereby contributing toward precision-based strategies for early prediction, prevention, and personalized management of periodontal disease.

3. Aims and Objectives

Aim: To study the host immunogenetic and microbiome specific diagnostic and therapeutic biomarkers; To understand the association between HLA and oral microbiome diversity in periodontitis.

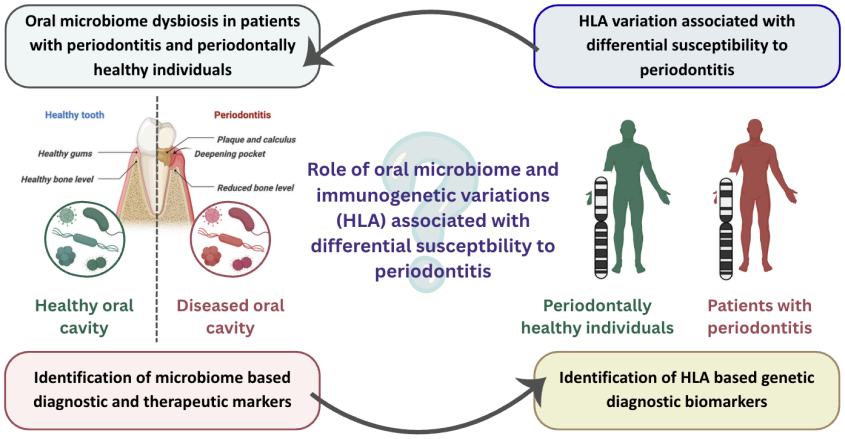
OBJECTIVE 1: Comparative genomic analysis of human oral microbial genomes to identify potential diagnostic and therapeutic markers

OBJECTIVE 2: To reveal the Immunogenetic variations associated with periodontitis by identifying potential HLA variants

OBJECTIVE 3: To study the salivary microbiome diversity, HLA genetic diversity and to correlate with differential susceptibility to periodontal disease

4. Hypothesis/ Research Question

In India, Research on the oral microbiome and immunogenetic variations is still in its early stages. More studies are needed to understand the association of oral microbial diversity and Immunogenetic variations with respect to disease susceptibility.


Oral microbiome dysbiosis in patients with periodontitis and periodontally healthy individuals
HLA variation associated with differential susceptibility to periodontitis
Healthy tooth
Periodontitis
Healthy gums
Healthy bone level
Plaque and calculus
Deepening pocket
Reduced bone level
Role of oral microbiome and immunogenetic variations (HLA) associated with differential susceptbility to periodontitis
Healthy oral cavity
Diseased oral cavity
Periodontally healthy individuals
Patients with periodontitis
Identification of microbiome based diagnostic and therapeutic markers
Identification of HLA based genetic diagnostic biomarkers

Research Question Or Hypothesis

Research Question: Is there an association between alteration in oral microbiota and HLA genetic variation in patients with periodontitis compared to healthy controls?

Research Hypothesis: There is an alteration in the oral microbiota and genetic variation of HLA allele in patients with periodontitis and healthy controls. Null Hypothesis: There is no significant alteration in the oral microbiota and HLA allele in patients with periodontitis and healthy controls

5. Brief Literature Review

5.1 Overview of the Oral Microbiome in Periodontal Health and Disease

The oral cavity harbours a complex and diverse microbial ecosystem, known as the oral microbiome, which is crucial for maintaining oral homeostasis and overall health. This community includes bacteria, archaea, fungi, and viruses, all of which play significant roles in maintaining oral and systemic health. In a healthy state, a balanced relationship exists among these microorganisms, supporting metabolic functions and immune responses. However, a disruption in this balance, termed microbial dysbiosis, is a key factor in the

development of oral diseases such as periodontitis and gingivitis [8]. Periodontitis is a highly prevalent inflammatory disease driven by dysbiosis, leading to progressive destruction of soft and hard periodontal tissues [1]. At the phylum level, healthy oral microbiota are typically dominated by *Firmicutes* (25-35%), *Proteobacteria* (15-25%), *Actinobacteria* (20-40%), *Bacteroidetes* (10-20%), and *Fusobacteria* (5-10%). In periodontitis, this balance shifts, with a relative increase in *Bacteroidetes* and *Spirochaetes* and a corresponding decline in *Firmicutes* and *Actinobacteria*. At the genus level, periodontitis shows increased relative abundance of *Porphyromonas, Treponema, Prevotella, Fusobacterium, and Filifactor,* which may together comprise 20-30% of the diseased biofilm, compared with <5–10% in health [9]. The shift to dysbiosis involves an overgrowth of certain pathogenic microbes and a loss of biodiversity.

5.2 HLA Genes and Immune Regulation in Periodontal Disease

The HLA system, which serves as the body's primary histocompatibility complex, plays a fundamental role in regulating immune responses against periodontitis and significantly influences an individual's susceptibility to, or resistance against, periodontitis [10]. Genetic variations within HLA genes are known to significantly impact the development and progression of both chronic periodontitis (CP) and aggressive periodontitis (AgP). Multiple investigations reported that specific Class I (HLA-A9, A24, B15, B18) and Class II (DR4) alleles tend to cluster with increased risk of early-onset and rapidly progressive periodontitis, whereas alleles such as HLA-A1, A2, and certain DRB haplotypes repeatedly appeared to confer protection. In South Indian cohorts, HLA-A24 and HLA-B15 were associated with aggressive periodontitis, whereas HLA-A9 showed a protective effect [11, 12]. Studies from East India (Bengali population) reported that HLA-B7, DR7, DR53, and DQ3 increased susceptibility to chronic periodontitis, while A1, A3, and Cw4 were found to be protective [13]. Indian studies reaffirm the role of HLA-A9, A24, and B15 in modulating risk for aggressive periodontitis, with consistent identification of A9 as a protective allele in certain populations, highlighting both overlap and divergence with international findings.

5.3 HLA-Microbiome Interactions in Periodontal Health and Disease

The interplay between HLA polymorphisms and the oral microbiome represents a critical determinant of periodontal health and disease [14]. Specific HLA alleles can influence how the host immune system recognizes and responds to oral microbial antigens, thereby shaping the composition and functional dynamics of the oral microbiome. In genetically susceptible individuals, certain alleles (HLA-DRB1*03, DRB1*15, DQB1*06) may present periodontal pathogen peptides inefficiently, resulting in suboptimal T cell activation, reduced mucosal IgG production, and impaired clearance of keystone pathogens [15]. Conversely, protective alleles enhance peptide presentation and immune surveillance, promoting the maintenance of commensal populations like *Streptococcus sanguinis* and *Veillonella* spp., preserving

microbial diversity, and limiting pathogen expansion. These differences highlight how HLA-mediated antigen presentation directly modulates susceptibility or resistance to dysbiosis-driven inflammation [16]. Overgrowth of pathogenic microbial complexes triggers pro-inflammatory cytokine release, activation of T-cell subsets, and chronic inflammation, leading to gingival barrier breakdown, connective tissue damage, and alveolar bone resorption [17].

Emerging evidence also suggests systemic interactions, including the "gut-oral axis," where HLA-mediated immune responses in the gut may influence oral microbial ecology and periodontal inflammation. The remarkable polymorphism of HLA genes plays a critical role in antigen presentation and host immune regulation, which can shape the composition of the oral microbiome by favoring or restricting the colonization of specific microbial taxa. Understanding this interplay between HLA diversity and the oral microbiome will provide important insights into host-microbe interactions and disease pathogenesis. Future studies integrating high-resolution HLA genotyping with deep metagenomic profiling are essential to elucidate these complex relationships and may ultimately support the development of personalized strategies for periodontal disease prevention and therapy.

6. Methodology

6.1 Methodology for Objective 1:

The study began with genome retrieval, where a total of 192 complete oral bacterial genomes were obtained from the National Center for Biotechnology Information (NCBI) database. These genomes were carefully selected to ensure completeness. Following retrieval, genome data preparation was carried out. The genomic sequences were systematically organized and annotated to ensure consistency and accuracy for downstream analyses. Subsequently, phylogenetic analysis was performed using both 16S rRNA gene sequences and whole-genome data. Phylogenetic trees were constructed to understand evolutionary relationships and clustering patterns among the bacterial species. To explore functional capabilities, functional gene prediction was conducted. This step involved identifying genes associated with antimicrobial resistance genes (ARGs), virulence factors, and heavy-metal resistance, providing insights into the pathogenic and adaptive potential of the organisms. Next, metabolic pathway analysis was undertaken to predict key biochemical pathways, particularly nitrate reduction and electron transport chain pathways, which are critical for bacterial survival and metabolic activity in the oral environment. Finally, all results were combined in a data integration and interpretation step. Comparative analysis across all bacterial genomes enabled the identification of patterns, correlations, and biologically meaningful insights into the structure and function of the oral microbiome.

6.2 Methodology for Objective 2 and Objective 3:

6.2.1 Sample Size:

The present analytical cross-sectional study was designed to collect and analyze a total of 58 saliva samples including healthy donors and patients with periodontitis. From the previous studies, Thomas et al. 2004 had found the HLA association with periodontitis. It was found that the HLA allele A9 and A24 frequency was 70% in patients with periodontitis and 30% in controls. With these proportions, 80% power, 95% confidence interval and 1:1 allocation ratio, the sample size was found to be 58 (29 in each group). Although the estimated sample size was 58 (29 per group), the number of samples was increased to 100 (50 per group) to improve the statistical strength of the study.

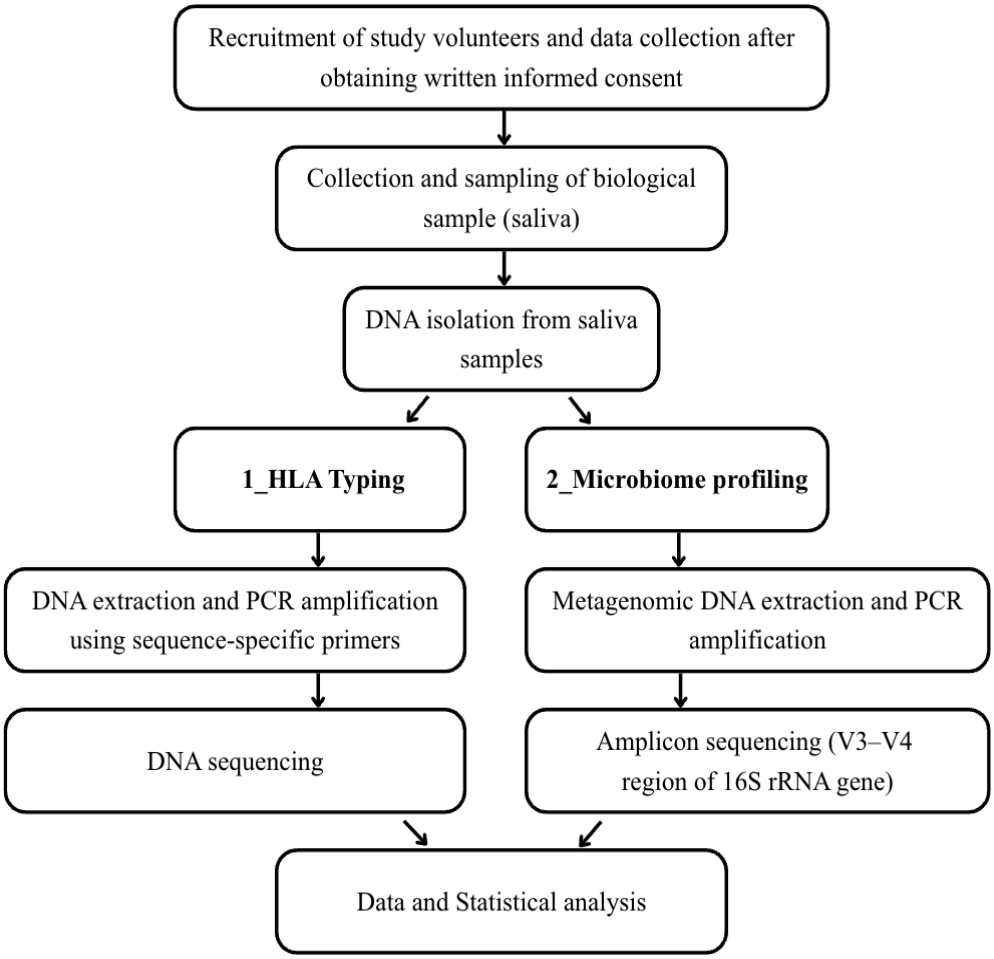
Participants were categorized into periodontally healthy and having periodontitis groups based on clinical criteria. Healthy individuals had no systemic disease, probing depth ≤2 mm, no clinical attachment loss (CAL), and no inflammation. Periodontitis cases included adults (≥18 years), characterized by ≥3 periodontal pockets with probing depth >5 mm, CAL ≥2 mm, and at least 16 natural teeth. Individuals with recent periodontal therapy, antibiotic use within the past 6 months, heavy smoking, or pregnancy/lactation were excluded to ensure consistency and minimize bias

This study was designed to investigate the relationship between host immunogenetic factors and oral microbiota composition in patients and healthy control using an observational and analytical approach. Following ethical approval, study participants were recruited, and written informed consent was obtained prior to data and sample collection.

6.2.2 Workflow:

Workflow summarizing the methodology for objectives 2 and 3 is shown in **Figure 1.** The generated sequencing data from both approaches were subsequently processed and analyzed using appropriate bioinformatics pipelines. Finally, statistical analyses were performed to identify associations between HLA types and variations in the oral microbiota composition and diversity, enabling comprehensive interpretation of host-oral microbe interactions.

Figure 1: Workflow summarizing the methodology for Objectives 2 and 3


Recruitment of study volunteers and data collection after obtaining written informed consent
Collection and sampling of biological sample (saliva)
DNA isolation from saliva samples
1_HLA Typing
2_Microbiome profiling
DNA extraction and PCR amplification using sequence-specific primers
Metagenomic DNA extraction and PCR amplification
DNA sequencing
Amplicon sequencing (V3–V4 region of 16S rRNA gene)
Data and Statistical analysis

7. Results and Discussion

7.1 Key Findings for Objective 1: "Comparative genomic analysis of human oral microbial genomes to identify potential diagnostic and therapeutic markers"

7.1.1 Retrieval and Annotation of Oral Bacterial Genomes

A total of 192 oral bacterial genomes were retrieved from the NCBI database. The species list was systematically sorted and curated to ensure inclusion of bacteria relevant to the oral microbiome **(Figure 2)**. The selected genomes were then subjected to annotation using the RAST server to identify functional features and gene content. The resulting annotated sequences were subsequently utilized for all downstream analyses. Species-wise distribution from the sorted data revealed notable variation in the abundance of complete genomes among the 41 oral bacterial species.

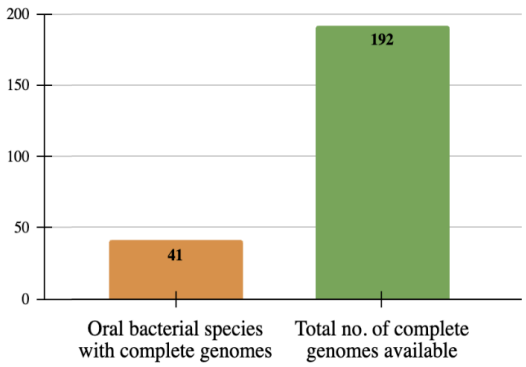
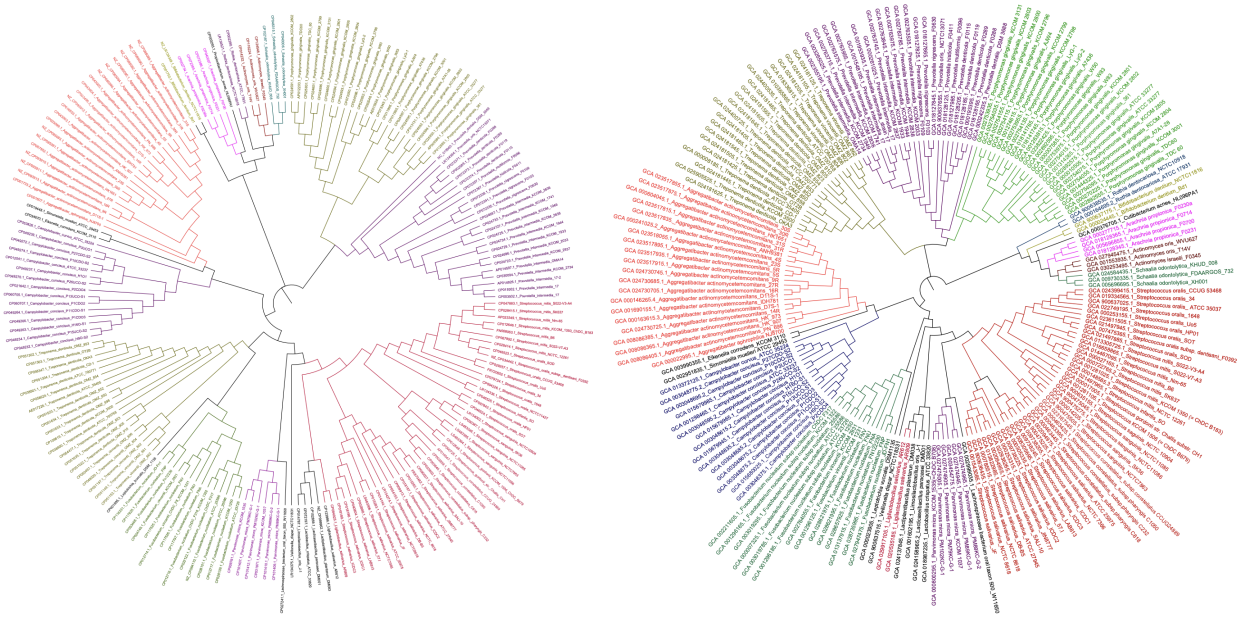


Figure 2: Retrieval of 192 complete oral bacterial genomes from the NCBI database

7.1.2 Phylogenetic Analysis

A comprehensive phylogenetic analysis was performed using 192 oral bacterial genomes, representing 41 species, based on 16S rRNA gene and whole genome sequences **(Figure 3).** The constructed phylogenetic tree demonstrated clear clustering patterns at the genus level, indicating strong evolutionary relationships among the analyzed taxa. Closely related species were observed to group together within the same branches, supporting the taxonomic consistency of the dataset.



(A) (B)

Figure 3: (A) 16S rRNA-based and (B) whole-genome-based phylogenetic trees of 192 oral bacterial genomes representing 41 species.

The overall tree topology revealed the presence of three major clusters, which were further subdivided into ten distinct sub-clusters, highlighting the diversity and hierarchical organization of the oral microbiota. The whole-genome-based phylogenetic analysis provided higher resolution, capturing finer evolutionary distinctions among taxa. This approach resulted in four major clusters and nine sub-clusters, suggesting improved discrimination of genomic diversity compared to 16S rRNA-based analysis. Together, these analyses highlight both the conserved phylogenetic relationships and the enhanced resolution of whole-genome approaches in characterizing oral microbial diversity.

7.1.3 Serendipitous Research Findings

Whole-genome phylogenetic analysis and comparison of genomic indices resulted in identification of few novel bacterial species that extended beyond the primary scope of the study. Through detailed comparative genomics of the analyzed dataset, we observed distinct genomic variations that did not align with previously characterized species. This led to the identification of six distinct oral bacterial species from validly published oral bacterial species *(Arachnia propionica, Parvimonas micra, Eikenella corrodens, tannerella forsythia, Fusobacterium nucleatum* and *Treponema denticola)* which include 4 novel species and reclassification of 2 species**.** A standardized comparative genomics workflow was established, integrating whole-genome phylogeny, ANI and dDDH-based species delineation, pan-genome and functional analyses, which enabled the accurate identification of novel oral bacterial species and reclassification of existing taxa **(Figure 4).** These findings highlight the power of comparative genomics not only in understanding known microbial taxonomy and functional diversity but also in uncovering previously misclassified taxa or reclassified species within the oral microbiota.

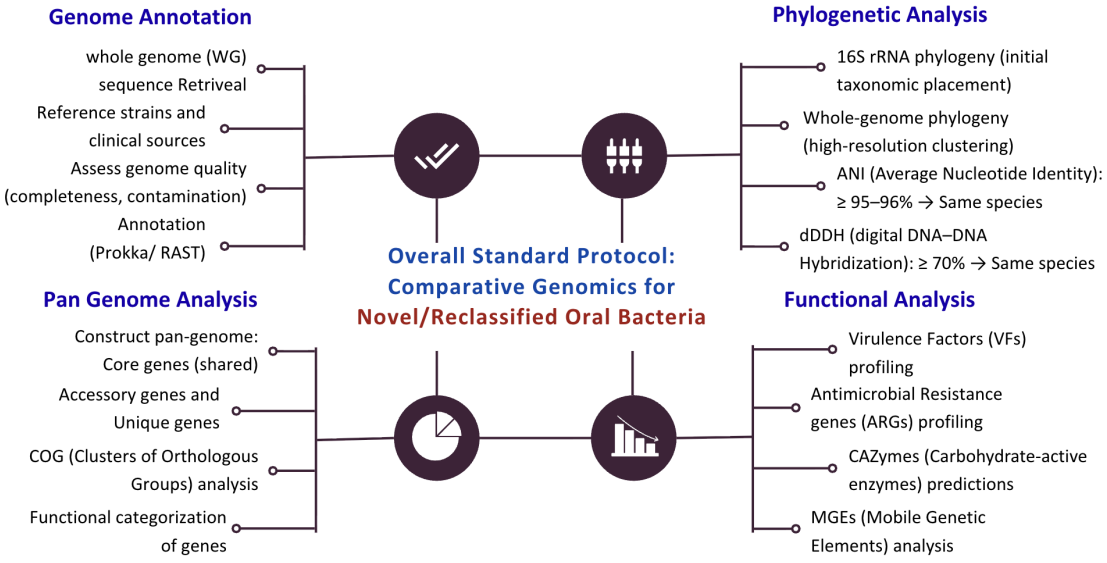

Genome Annotation
whole genome (WG) sequence Retriveal
Reference strains and clinical sources
Assess genome quality (completeness, contamination)
Annotation (Prokka/ RAST)
Phylogenetic Analysis
16S rRNA phylogeny (initial taxonomic placement)
Whole-genome phylogeny (high-resolution clustering)
ANI (Average Nucleotide Identity): ≥ 95–96% → Same species
dDDH (digital DNA–DNA Hybridization): ≥ 70% → Same species
Overall Standard Protocol: Comparative Genomics for Novel/Reclassified Oral Bacteria
Pan Genome Analysis
Construct pan-genome: Core genes (shared)
Accessory genes and Unique genes
COG (Clusters of Orthologous Groups) analysis
Functional categorization of genes
Functional Analysis
Virulence Factors (VFs) profiling
Antimicrobial Resistance genes (ARGs) profiling
CAZymes (Carbohydrate-active enzymes) predictions
MGEs (Mobile Genetic Elements) analysis

Figure 4: Overall standard protocol: Comparative genomics for novel/reclassified oral bacteria

7.1.4 Virulence Factor Profiling Across Oral Bacterial Species

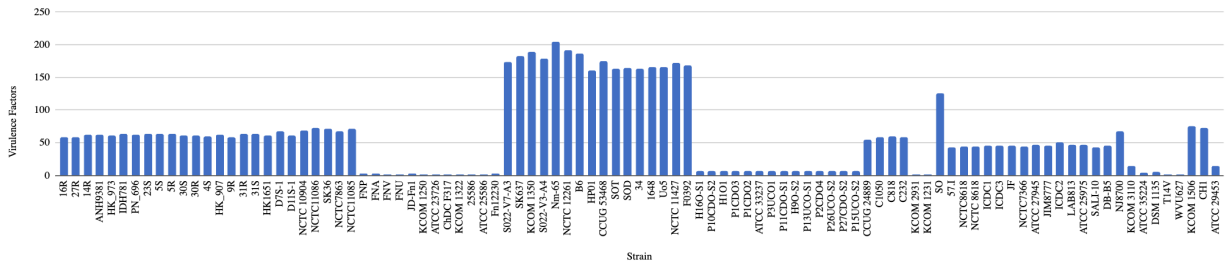


Figure 5: Species-level variation in virulence factor counts among 192 oral bacterial species

Distribution of virulence factors (VFs) across oral bacterial species was analyzed using the PATRIC database **(Figure 5)**. The analysis revealed distinct variations in virulence potential among species. Species such as *Streptococcus mitis* and *Streptococcus oralis* exhibited high virulence factors with counts varied from 174-205 and 160-175 respectively, indicating a substantial repertoire of genes associated with pathogenicity. Moderate virulence factor counts were observed in *Streptococcus sanguinis* (68-73 VFs), *Aggregatibacter actinomycetemcomitans* (59-68 VFs), and *Streptococcus gordonii* (72-75 VFs). As dksA gene is present across pathogenic organisms which is a transcriptional regulatory protein that interacts with RNA polymerase, and its inhibition may simultaneously disrupt multiple virulence pathways. Also targeting clpB, chaperone systems could weaken survival of bacterial pathogens without directly inducing strong resistance.

7.1.5 Species-Level Patterns of Antimicrobial Resistance Genes

Species-level analysis of ARGs, predicted using the PATRIC database, revealed diverse resistance mechanisms across oral bacterial species. Key resistance determinants included efflux pump systems such as EmrAB-TolC, MacA, MacB, and CmeABC; ribosomal protection proteins and target site mutations including Tet(M), Tet(L), tetQ, rpsJ, and rpsL; and β-lactam resistance-associated penicillin-binding proteins (PBPs) such as PBP2x, PBP2b, PBP1a, and pbpX. Additionally, quinolone resistance genes (gyrA, gyrB, parC, parE) and vancomycin resistance-related genes (VanO-type, BceR) were identified, indicating a broad spectrum of resistance strategies.

7.1.5.1 Distribution of Acquired and Intrinsic ARGs

Analysis of ARGs among oral bacterial genomes revealed distinct patterns of prevalence **(Figure 6)**. Widespread resistance traits included copper homeostasis (100%) and copper tolerance (90%), indicating strong intrinsic metal resistance. Moderately prevalent features comprised cobalt-zinc-cadmium resistance (92%) and multidrug efflux pumps (80%), suggesting a broad capacity for resistance and environmental

adaptation. Limited presence was observed for cadmium resistance (31%) and beta-lactamase genes (37%). In contrast, rare resistance determinants such as zinc resistance (4%), streptothricin resistance (4%), vancomycin resistance (1%), and streptolysin S biosynthesis (1.5%) were minimally distributed. Overall, these findings highlight a dominance of intrinsic resistance mechanisms, with selective acquisition of clinically relevant resistance genes.

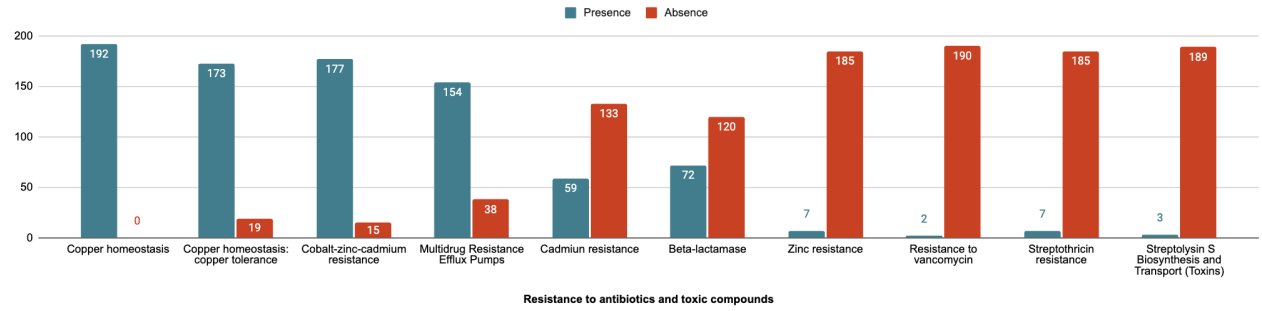


Figure 6: Acquired and natural ARGs across genomes

7.1.6 Genomic Distribution of Denitrification and Nitrogen Metabolism Pathways

Denitrification pathway analysis revealed limited distribution among oral bacterial genomes, with denitrification gene clusters present in only 4.2% (8/192) and denitrifying reductase gene clusters in 13% (25/192) of genomes, indicating a restricted role in nitrogen metabolism **(Figure 7A).** Analysis of additional nitrogen metabolism pathways revealed variable distribution across genomes **(Figure 7B).** Moderate prevalence of genes associated with nitrosative stress (16%) and nitrate/nitrite ammonification (33%) observed among oral bacterial genomes suggesting their potential role in microbial adaptation and nitrogen metabolism. In contrast, ammonia assimilation and allantoin utilization pathways were rare, showing very low occurrence across genomes. These findings suggest selective utilization of nitrogen metabolism pathways among oral bacterial species.

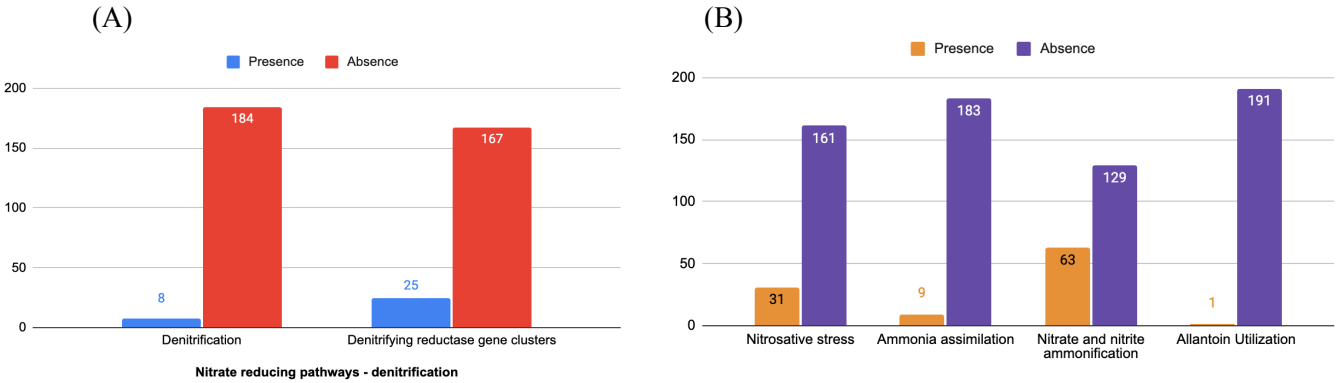


Figure 7: Distribution of (A) denitrification and (B) associated nitrogen metabolism pathway genes

7.1.7 Comparison of Pathogenic, Opportunistic, and Beneficial Oral Bacteria

Oral bacterial species were categorized into pathogenic, opportunistic pathogens, and beneficial groups based on literature evidence to assess their functional roles within the oral microbiome. The distribution showed a higher number of beneficial species (n=16) compared to pathogenic (n=13) and opportunistic pathogens (n=12), indicating a predominance of health-associated microbes. Notably, genes associated with nitrate-reducing pathways were absent in pathogenic oral bacterial genomes, suggesting limited contribution to beneficial nitrogen metabolism, whereas beneficial bacteria may play a role in maintaining microbial balance and oral health.

Comparative genomic analysis of 192 oral bacterial genomes representing 41 species revealed genus-level clustering and clear species-level relationships, enabling identification of novel oral bacterial candidates from validly published species. The discovery of novel oral taxa (e.g., within the genera *Arachnia, Parvimonas, Eikenella,* and *Tannerella*) in the present study highlights the vast "hidden diversity" of the oral microbiota, an issue widely emphasized in foundational 16S rRNA based studies for identification of bacterial species [18].

7.2 Key Findings for Objective 2: "To reveal the Immunogenetic variations associated with periodontitis by identifying potential HLA variants"

7.2.1 Primer Details for HLA-Based Genotyping

A total of 10 primers were used for HLA-based genotyping, selected from previously validated literature. The primers were optimized based on annealing temperature and expected amplicon size. The targeted alleles included HLA-A (A10, A24/A9, A1, A3), HLA-B (B7, B15:01, B15), HLA-C (CW4), and class II genes HLA-DR7 and HLA-DQ3, with amplicon sizes ranging from 122 to 1023 bp and annealing temperatures between 53°C and 62°C. Sequencing validation was performed for all primers to confirm accurate amplification, supported by gel electrophoresis, Chromas visualization and BLASTn analysis; a representative result from one primer (HLA-A10) is presented **(Figure 8).**

7.2.2 HLA-A10 Sequence Validation

Primer specificity for HLA-based genotyping was validated by sequencing one representative amplicon from each primer set, confirming the amplification of the corresponding target HLA allele. PCR was performed under optimized conditions (1% agarose gel, 57 °C annealing) **(Figure 8).** Sequence quality was assessed using Chromas and verified through alignment with NCBI BLASTn, showing high identity with HLA sequences. The validated primers were subsequently used for genotyping across 100 samples.

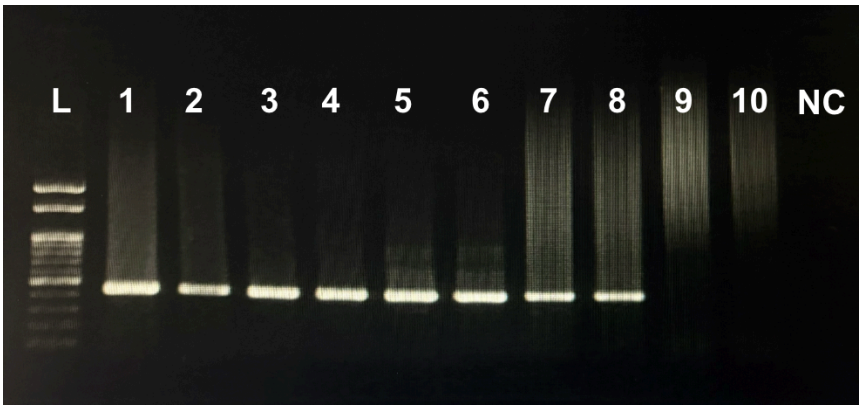
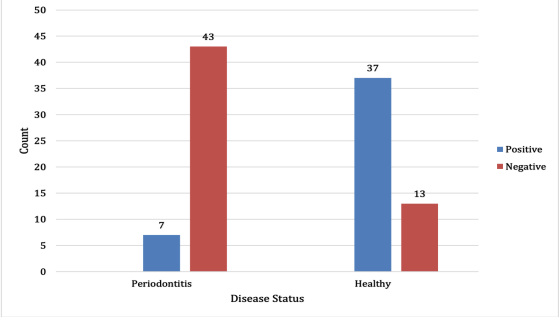


Figure 8: The gel image shows successful amplification of HLA alleles, indicated by clear bands at ~442 bp. 1–10: Diseased samples; L: DNA ladder; NC: Negative control

7.2.3 Association of HLA Alleles with Periodontitis and Healthy Status

HLA-B15:01 Association with Periodontitis



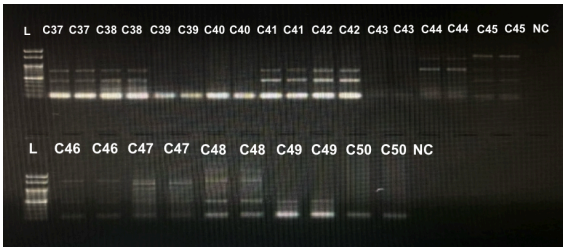


Figure 9: Representative gel image showing amplification of HLA-B15:01 allele from healthy control Samples (L-100bp DNA Ladder, samples: C37- C50 with duplicates)

Statistical test: Chi-square test;

Periodontitis group: 7 positive, 43 negative;

Healthy group: 37 positive, 13 negative;

Statistics: $\chi^2 = 36.526$, $p < 0.001$;

Odds Ratio: 0.057 (95% CI: 0.021–0.158).

Conclusion: Presence of HLA-B15:01 may be protective and associated with reduced susceptibility to periodontitis.

Table 1: Association of HLA alleles with periodontitis and healthy status

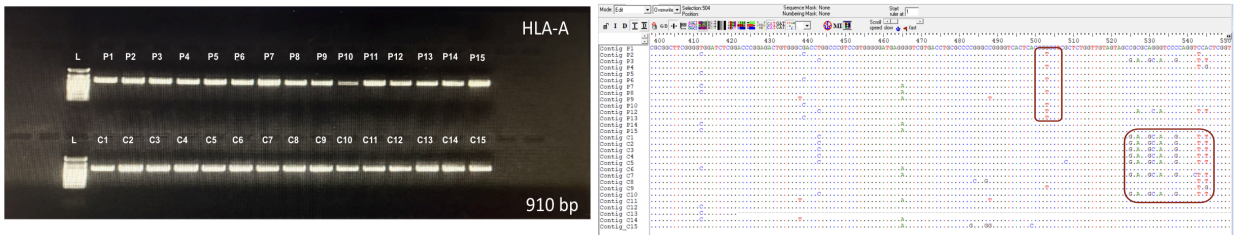
HLA Allele	Status	Periodontitis	P (%)	Healthy	H (%)	Chi-square	p-value	Risk (Odds Ratio)
A10	Positive	34	68%	38	76%	0.794	0.373	0.671
	Negative	16	32%	12	24%			
B15:01	Positive	7	14%	37	74%	36.526	**<0.001**	0.057
	Negative	43	86%	13	26%			
A9/A24	Positive	27	54%	34	68%	2.060	0.151	0.552
	Negative	23	46%	16	32%			
DR7	Positive	20	40%	33	66%	6.784	**0.009**	0.343
	Negative	30	60%	17	34%			
CW4	Positive	16	32%	12	24%	0.794	0.373	1.490
	Negative	34	68%	38	76%			
DQ3	Positive	18	36%	12	24%	1.714	0.190	1.781
	Negative	32	64%	38	76%			
B7	Positive	35	70%	24	48%	5.002	**0.025**	2.528
	Negative	15	30%	26	52%			
A1	Positive	15	30%	20	40%	1.099	0.295	0.643
	Negative	35	70%	30	60%			
A3	Positive	5	10%	13	26%	8.889	**0.003***	0.077
	Negative	10	20%	2	4%			
B15	Positive	0	0%	4	8%	4.615	0.1*	1.364
	Negative	15	30%	11	22%			

A p-value < 0.05 was considered statistically significant.

*Because the expected cell counts were below five, Fisher's exact test was used.

7.2.4 Sanger Sequencing-Based HLA Genotyping of HLA-A and HLA-DR Alleles

The HLA-A and HLA-DR primers used in this study are locus-specific, conserved primers designed to target highly conserved regions, enabling amplification across multiple alleles. A total of 60 samples were selected for HLA genotyping, comprising 30 samples each for HLA-A and HLA-DR allele groups. Sanger sequencing was performed using HLA-specific forward and reverse primers (n=30 each for health and disease group) with an annealing temperature of 62 °C. The targeted loci included HLA-A (Class I) and HLA-DR (Class II) genes. The same set of samples was used for both HLA genotyping and oral microbiome analysis. Successful amplification of the exon 2 region of HLA-A and HLA-DR genes was confirmed by the presence of clear bands in gel electrophoresis **(Figure 10A).**



(A) (B)

Figure 10: (A) PCR amplification of exon 2 regions of HLA-A and -DR genes (B) Multiple sequence alignment of the exon 2 region of HLA-A of study samples. Red box highlighting nucleotide variations at specific positions.

7.2.5 Sequencing and Alignment of the HLA-A Exonic Region

Multiple sequence alignment of the exonic region of HLA-A revealed a high degree of conservation across samples, with distinct nucleotide variations at specific positions (~503 and ~525-545 bp), indicating allelic differences **(Figure 10B).** These variations were localized and consistent among subsets of samples, while no major insertions or deletions were detected. Overall, the alignment confirms successful sequencing and highlights allelic variability within the exonic region of HLA-A. Multiple sequence alignment of the HLA-DR exonic region revealed a high degree of conservation across all samples, with no notable nucleotide variations observed between periodontitis and healthy groups.

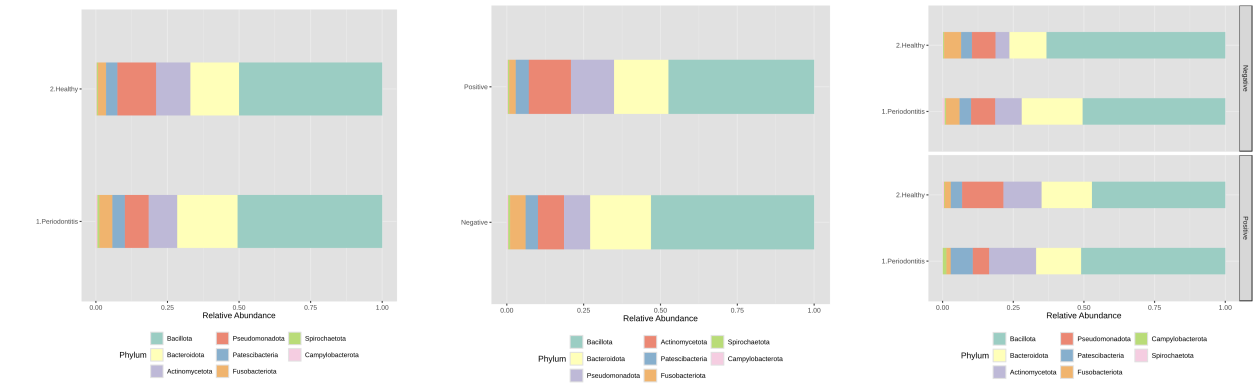
While microbial dysbiosis initiates periodontal destruction, the present study confirms that host's immunogenetic makeup significantly dictates disease susceptibility. Identification of HLA-B7 as a risk factor, alongside HLA-B15:01, HLA-DR7, and HLA-A3 as protective factors, aligns with the established results that HLA polymorphisms determine the efficiency of T-cell antigen presentation. Conversely, while Stein et al., 2008 identified HLA-B15 as a distinct susceptibility factor in Caucasian population, the current study found no significant association for HLA-B15, underscoring the consensus that specific HLA allelic associations are highly dependent on geographical, ethnic, and population variations [19].

7.3 Key Findings for Objective 3: "To study the salivary microbiome diversity, HLA genetic diversity and to correlate with differential susceptibility to periodontal disease"

A total of 30 samples (15 per group - periodontitis and healthy) were analyzed to study salivary microbiota diversity and its association with HLA status and periodontal disease susceptibility. Next-generation sequencing generated a total of ~2.12 million high-quality reads from all samples (n=30), with read counts ranging from ~6,252 to 236,809 per sample. The high quality reads were used for the prediction of Operational Taxonomic Units (OTUs) and downstream analysis of taxonomic abundance and diversity of the oral microbiota.

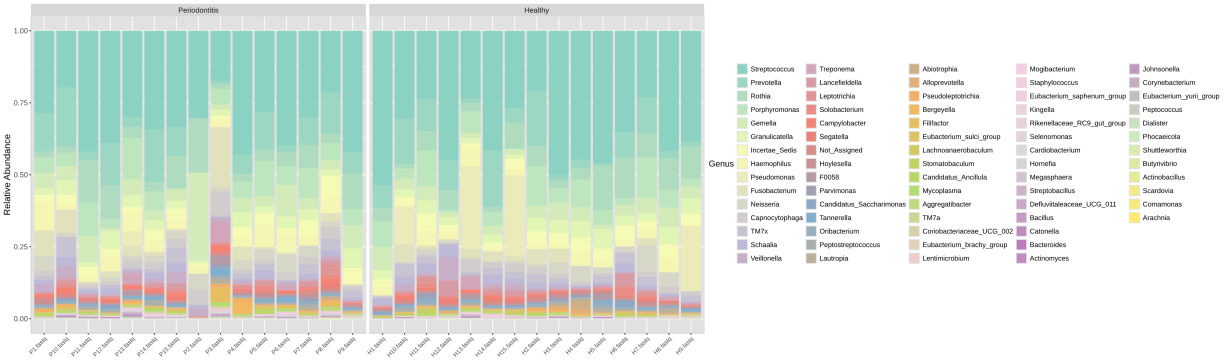
7.3.1 Relative Abundance of Oral Microbial Diversity

Relative abundance of oral microbial taxa was compared between groups based on disease status and HLA status. Analysis highlights differences in microbial composition and diversity between groups at both phylum and genus level **(Figure 11).**



(A) (B) (C)

Figure 11: Relative abundance of oral microbiota at phylum level based on (A) disease status, (B) HLA status, and (C) combined disease and HLA status.



(D) Relative abundance of oral microbiota at genus level based on disease status

7.3.2 Alpha Diversity Analysis at Species Level

Significant differences in oral microbial diversity were observed for both periodontal disease and HLA-B15:01 status, as determined by the Wilcoxon test (periodontitis: $p = 0.0073$; HLA status: $p = 0.0097$), indicating the association with alterations in the oral microbiota. Alpha diversity at species level showed a significant difference across groups (Kruskal-Wallis test, $p = 0.04$) **(Figure 12)**. Pairwise comparison revealed a significant difference between Periodontitis-HLA-B15:01 Absent and Healthy-HLA-B1501 Present groups ($p = 0.008$). These findings suggest that HLA-B15:01 status may influence microbiome diversity in relation to periodontal disease.

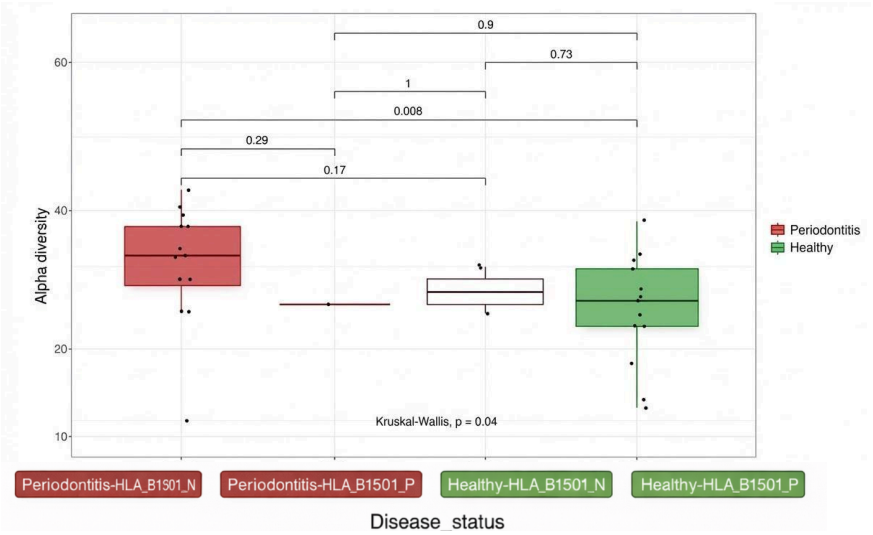
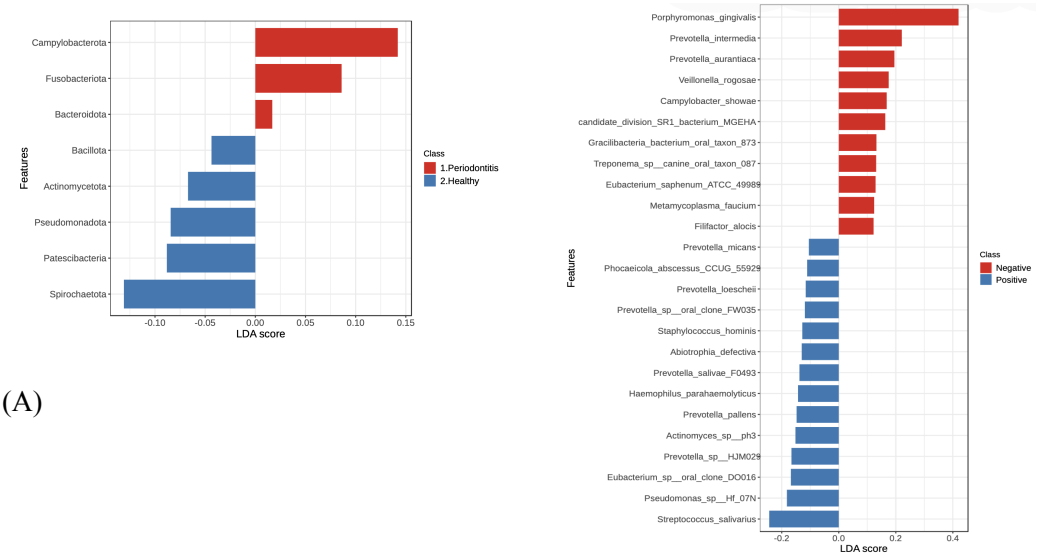


Figure 12: Species-level alpha diversity of oral microbiota between groups stratified by periodontitis status and HLA-B15:01 status.

7.3.3 Differentially Abundant Phyla and Species Identified by LEfSe Analysis

LEfSe analysis at the genus and species levels identified differential microbial signatures associated with disease status **(Figure 13A)** and HLA status respectively **(Figure 13B)**. Notably, *Porphyromonas gingivalis* showed increased abundance in the HLA-B15:01-negative group, suggesting a higher burden of this periodontal pathogen in the absence of the allele.



(A) (B)

Figure 13: LEfSe analysis showing differentially abundant phyla (A) between periodontitis and healthy groups and differentially abundant species (B) between HLA status

7.3.4 Association of *P. gingivalis* with Disease and HLA-B15:01 Status (MaAsLin2 Analysis)

MaAsLin2 analysis revealed a significant association of *P. gingivalis* with both disease and HLA-B15:01 status **(Figure 14).** A negative log2 fold change indicated higher abundance in periodontitis samples and in HLA-B15:01 negative individuals **(Table 2).** This association remained statistically significant after multiple testing corrections (FDR < 0.05), further supporting a strong negative association.

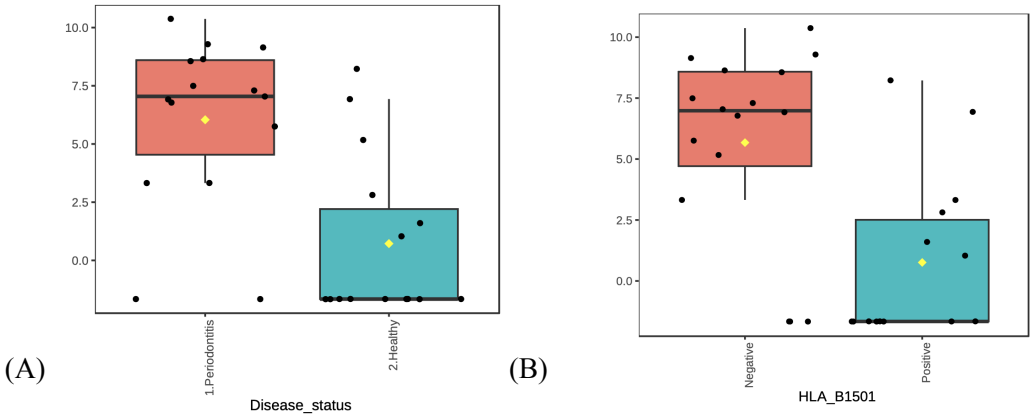


Figure 14: Boxplot showing MaAsLin2 analysis showing the association of *P. gingivalis* with (A) Periodontitis status and (B) HLA-B15:01 Status

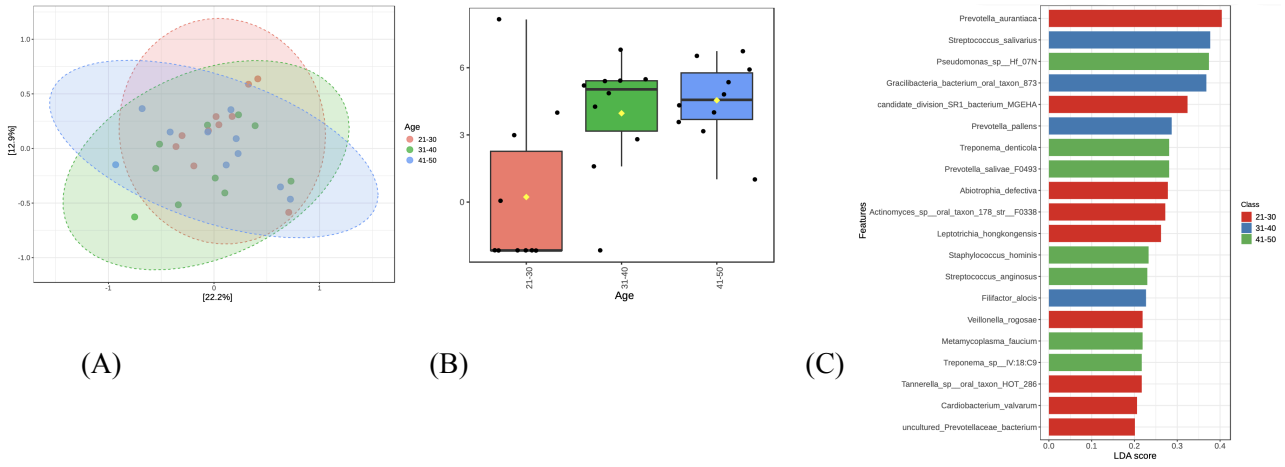
Table 2: Association of *P. gingivalis* with Periodontitis and HLA-B15:01 status

Porphyromonas_gingivalis	**Log2FC**	**St.Error**	**P-value**	**FDR**
For Disease status	+6.09	1.4	0.000159	0.00891
For HLA-B15:01 status	-5.75	1.45	0.000475	0.0133

The findings suggest that the absence of HLA-B15:01 may be associated with increased susceptibility to periodontitis by allowing colonization or enrichment of key pathogens such as *P. gingivalis*, highlighting a possible host-microbiome interaction.

7.3.5 Age-Associated Variations in Oral Microbial Community Structure and Composition

Beta diversity analysis using PCoA revealed significant differences in microbial community structure across age groups (p = 0.013), indicating age-associated variation in the oral microbiota. Further analysis using LEfSe and MaAsLin2 identified differentially abundant taxa linked to age, with *Metamycoplasma faucium* significantly enriched in the 41-50 age group **(Figure 15).**



(A) (B) (C)

Figure 15: Age-associated differences in oral microbiota diversity and taxonomic abundance. (A) Beta-diversity analysis (B) LEfSe analysis and (C) MaAsLin2 Analysis of *Metamycoplasma faucium*

7.3.6 Quantification of *P. gingivalis* using Real-time PCR

To further validate the 16S rRNA amplicon sequencing-based findings, the abundance of *P. gingivalis* was quantified using real-time PCR **(Table 3).** Lower Ct values indicate higher bacterial load. The periodontitis group showed lower Ct values compared to the healthy group, indicating higher abundance of *Porphyromonas gingivalis* in periodontitis patients and supporting its strong association with periodontal disease.

Table 3: Comparison of salivary *P. gingivalis* Ct values between healthy and periodontitis (N=100)

Disease Status	n	Mean Ct	SD	Mean Rank	Sum of Ranks	Mann – Whitney U	p-value	Effect size
Healthy	50	25.736	6.312	64.84	3242	1967.00	< 0.001	0.574
Periodontitis	50	20.000	3.677	36.16	1808			

7.3.7 Salivary *Porphyromonas gingivalis* Load Across Periodontitis Severity

Salivary abundance of *P. gingivalis* was quantified using real-time PCR, revealing a significant difference across disease severity groups (Kruskal-wallis, $H = 19.964$, $df = 2$, $p < 0.001$)**.** The effect size was moderate to large ($\varepsilon^2 = 0.407$; 95% CI: 0.251-0.580), indicating a substantial association. Notably, samples from severe periodontitis exhibited the lowest Ct values, corresponding to higher bacterial abundance. To determine the pairwise differences between severity groups, Dunn's post-hoc test with Bonferroni correction was performed **(Figure 16, Table 4).**

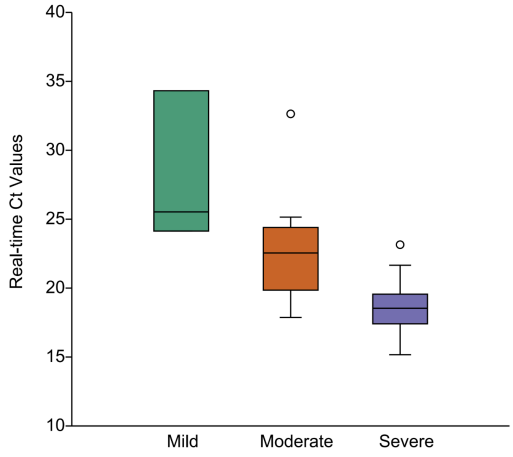


Table 4: Dunn's post-hoc comparisons of salivary *P. gingivalis* Ct values

Comparison	z	p	Bonferroni - adjusted p
Mild vs Moderate	1.047	0.295	0.295
Mild vs Severe	3.168	0.002	0.005
Moderate vs Severe	3.547	<0.001	0.001

Figure 16: Distribution of salivary *P.gingivalis* Ct values across periodontitis severity groups

qPCR validation confirmed higher bacterial load (lower Ct values) in periodontitis samples, with progressive increase in abundance in severe disease, strengthening the oral microbial association between *P gingivalis* and disease severity. Integrating HLA and microbiome data suggest that HLA-B15:01 may confer a protective effect, likely by enhanced $CD8^+$ T-cell-mediated immune response against *P. gingivalis*, thereby preventing dysbiosis and disease progression.

The absence of the protective HLA-B15:01 allele was associated with a significant enrichment of the salivary keystone pathogen *P. gingivalis.* This host-mediated restriction of pathogens supports the concept that an individual's immunogenetic profile actively shapes their microbial community structure. This directly mirrors the mechanisms observed by Reichert et al. (2013), who demonstrated that specific host alleles (e.g., HLA-DRB1*04) independently decrease the colonization risk of other key pathogens like Aggregatibacter actinomycetemcomitans [20].

8. Summary and Conclusion

Comparative genomics of 192 oral bacterial genomes revealed substantial genetic diversity and clear genus-level clustering, enabling the identification of novel species and the reclassification of existing oral bacteria. Integrated functional and interaction analyses further identified key virulence factors, including *dksA* and *clpB*, along with important metabolic adaptations, highlighting their potential as diagnostic and therapeutic targets. HLA typing based immunogenetic analysis demonstrated significant associations with specific HLA alleles, including HLA-B15:01, HLA-DR7, HLA-B7, and HLA-A3, underscoring the role of host immunogenetic variability in susceptibility to periodontal disease. Moreover, integrated analysis of the oral microbiota and HLA profiles revealed that host immunogenetic variation is significantly

associated with oral microbial diversity. Notably, HLA-B15:01 emerged as a key immunogenetic mediator that modulates oral microbiota composition by limiting the colonization of *Porphyromonas gingivalis*, maintaining microbial balance, and thereby conferring protection against periodontitis. Overall, the findings confirm a strong association between oral microbiota composition, diversity and HLA based host immunogenetic variations, providing molecular support for the polymicrobial synergy and dysbiosis (PSD) model of periodontitis. The identification of novel species and intrinsic ARGs expands current knowledge of periodontal microbiology. The integration of oral microbiota profiling with HLA genotyping suggests a promising dual-biomarker approach for personalized diagnosis, targeted therapeutic interventions, and improved disease management.

9. Limitations

- Samples were collected from a single tertiary care hospital, limiting geographic diversity/ethnicity and generalizability across India.
- In silico predictions of virulence factors, ARGs, and pathways lack experimental validation.
- Microbiome analysis was conducted on a relatively small subset (n = 30) using amplicon sequencing of 16S rRNA, which may limit statistical power and broader population representation.

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11. Publications

1. **Suresh R**, Jayachandiran S, Balu P, Ramasamy D. Comparative genomics reveals genetic diversity and differential metabolic potentials of the species of *Arachnia* and suggests reclassification of *Arachnia propionica* E10012 (=NBRC_14587) as a novel species. ***Archives of Microbiology***. 2025 Mar 18;207(4):93. (**First Author**)

2. **Suresh R**, Jayachandiran S, Balu P, Ranganadin P, Dhamodharan R. Comparative genome analysis of human pathogen *Parvimonas micra* revealed strain JM503A as a potential novel species in the genus *Parvimonas* & high intra-species functional diversity. ***Microbial Genomics.*** 2025 Sep 22. (**First Author**)

3. **Suresh R**, Jayachandiran S, Balu P, Ramasamy D. Comparative and Pan-Genome Analysis of the Complete Genomes of the Periodontal Pathogen *Tannerella forsythia* Reveals Unique Tetracycline Resistance Protein Q and GT22 Carbohydrate-Active Enzyme Family in Strain 92A2. ***Ecological Genetics and Genomics.*** 2025 Nov 17:100428. (**First Author**)

4. **Suresh R**, Jayachandiran S, Balu P, Ramasamy D. Comparative and Phylogenomic Analysis of the *Eikenella* Genus Suggests Taxonomic Reclassification of *Eikenella corrodens* Strain KCOM 3110 as a Novel Species and Reveals Unique CE1 Carbohydrate-Active Enzyme Family. ***Molecular Genetics and Genomics*** 2026 Dec;301(1):20. (**First Author**)

5. Jayachandiran S, **Suresh R,** Dhamodharan R. Comparative and phylogenomic analysis of *Chlamydia pneumoniae* reveals unique carbohydrate-active enzyme family (GT5) among respiratory isolates. ***Infection, Genetics and Evolution***. 2025 Aug 21:105813. (**Co-Author**)

6. Jayachandiran S, **Suresh R,** Ramasamy D. Bacteriome in Health & Diseases, 'Textbook of Bacterial Zoonoses" ***Book chapter, Springer Nature.*** (**Co-Author**) - Accepted (In Press)

12. Podium and Poster Presentations

- International Conference on Emerging and Re-emerging Infectious Diseases (ICER-ID 2025) - presented work on oral microbiome resistome profiling.
- International Conference on Pathology & Genomics for Precision Medicine (PAGE-25) - presented work on comparative genomics.
- 6th MICROSUMMIT 2025 International Conference - presented work on infectious disease genomics and antimicrobial resistance.

Molecular Genetics and Genomics (2026) 301:20
https://doi.org/10.1007/s00438-025-02342-7

ORIGINAL ARTICLE

Comparative and phylogenomic analysis of *Eikenella* genus suggest taxonomic reclassification of *Eikenella corrodens* strain KCOM 3110 as novel species and reveals unique CE1 carbohydrate-active enzyme family

Roja Suresh[1] · Susanthika Jayachandiran[1] · Pratebha Balu[2] · Dhamodharan Ramasamy[1]

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Abstract

The *Eikenella* genus includes opportunistic pathogens of clinical importance, commonly associated with infections such as endocarditis, periodontitis, wound abscesses, and respiratory tract infections; however, its taxonomy, genetic diversity, and evolutionary relationships remain poorly understood. This study aims to clarify the genomic diversity and taxonomic boundaries within the genus through comprehensive comparative and phylogenomic analyses. We conducted a genomic comparison of 63 *Eikenella* genomes, including 35 high-quality drafts and 5 complete genomes, to understand their phylogenetic relationships and identify potential taxonomic misclassifications. Notably, *Eikenella corrodens* strain KCOM 3110 had the largest genome (2.46 Mb), the highest number of CDSs (2,877), and carried 611 unique genes among complete *Eikenella* strains. Phylogenetic trees based on 16S rRNA and whole genome data showed that KCOM 3110 did not cluster with the *Eikenella corrodens* group, but was closer to *Eikenella halliae*. The average nucleotide identity (~93%) and digital DNA-DNA Hybridization (~51.7%) values with *Eikenella corrodens* were below the species-level thresholds. Importantly, unique genes for antimicrobial resistance, metal tolerance, phage functions, and a distinct CE1 CAZyme profile supported its novelty. Overall, these findings indicate that *Eikenella corrodens* strain KCOM 3110 represents a novel species within the *Eikenella* genus. The study highlights the power of genome-based approaches in resolving bacterial taxonomy. It provides new insights into the evolutionary and functional diversity of *Eikenella* while laying the foundation for future studies on the ecological role, pathogenic potential, and clinical significance of this novel species.

Keywords Comparative genomics · CE1 Carbohydrate-active enzyme family · *Eikenella* genus · *Eikenella corrodens* · Species delineation · Taxonogenomics

Introduction

Eikenella is a genus of Gram-negative, facultatively anaerobic, non-motile bacilli that belong to the family *Neisseriaceae* (Jackson and Goodman 1972; Li et al. 2022). The type species, *Eikenella corrodens (E. corrodens)*, was first isolated from a human bite wound and was initially classified as *Bacteroides corrodens* before it was reclassified into its own genus in 1972 (Jackson and Goodman 1972). *E. corrodens* is a frequent coloniser of the human oral cavity, upper respiratory tract and gastrointestinal tract (Chen et al. 1989; Joshi et al. 1991; Liang et al. 2024). Although generally considered a commensal, it can act as an opportunistic pathogen and has been implicated in infections similar to those caused by the HACEK (*Haemophilus*, *Aggregatibacter*,

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MICROBIAL GENOMICS

RESEARCH ARTICLE

Suresh *et al.*, *Microbial Genomics* 2025;11:001511

DOI 10.1099/mgen.0.001511

MICROBIOLOGY SOCIETY

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Comparative genome analysis of human pathogen *Parvimonas micra* revealed strain JM503A as potential novel species in the genus *Parvimonas* and high intra-species functional diversity

Roja Suresh[1], Susanthika Jayachandiran[1], Pratebha Balu[2], Pajanivel Ranganadin[3] and Ramasamy Dhamodharan[1,*]

Abstract

Parvimonas micra is a Gram-positive, anaerobic bacterium commonly found in the oral cavity, skin and gastrointestinal tract. While typically a harmless organism, it can cause infections in individuals with weakened immune systems, leading to conditions like periodontitis and deep-tissue abscesses. This study focuses on the comparative genomic analysis of *P. micra* to explore its evolutionary relationships, antimicrobial resistance profiles and functional diversity by assessing phylogenetic analyses, resistance genes, virulence factors, mobile genetic elements, carbohydrate-active enzymes and pan-genome analysis. Comparative genomic analysis of 11 *P. micra* strains reveals significant functional variations among the strains, indicating notable interspecies diversity. Phylogenetic and comparative genome analysis revealed that strain JM503A is taxonomically distinct from the *P. micra* species, with genome similarity ranging from 54% to 61%. The 16S rRNA sequence similarity of strain JM503A is 98.28%, indicating a distinct phylogenetic position. The average nucleotide identity value ranging from 91.32% to 91.7% and digital DNA–DNA hybridization values ranging from 43.00% to 44.00% of JM503A with other strains are below the cutoff values <95% and <70%, respectively, which confirms JM503A as a novel species. Based on its evolutionary relationships, strain JM503A is identified as a potential new species of *Parvimonas*, providing important evidence for its reclassification as a new species within the genus *Parvimonas*.

Impact Statement

Parvimonas micra plays a significant role in human health, particularly in oral and systemic infections, as it is often linked to chronic periodontitis, contributing to gum inflammation and tissue destruction. In immunocompromised individuals, *P. micra* can lead to more severe infections such as abscesses, endocarditis and osteomyelitis. *P. micra* can also lead to systemic infections by entering the bloodstream and forming biofilms that resist treatment. Recognizing proper taxonomic identification and functional diversity of this pathogen is essential for effective management of both oral and overall health. The dearth of comparative genomic studies on this species in existing literature motivated us to investigate the taxono-genomic and

[*Continued on next page*]

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Keywords: antimicrobial resistance; comparative genomics; novel species; pan-genome analysis; *Parvimonas micra*; phylogenetic analysis; virulence factors.

Abbreviations: AMR, antimicrobial resistance; ANI, average nucleotide identity; BLASTN, Basic Local Alignment Search Tool (nucleotide); BPGA, Bacterial Pan Genome Analysis; CAZyme, carbohydrate-active enzyme; CDS, coding sequence; CE, carbohydrate esterase; COG, clusters of orthologous gene; CRC, colorectal cancer; dDDH, digital DNA–DNA hybridization; DDH, DNA–DNA hybridization; GCF, gingival crevicular fluid; Gegenees, genome evolutionary genealogy employing ensemble statistics; GGDC, Genome-to-Genome Distance Calculator; GH, glycoside hydrolase; GT, glycosyltransferase; MEGA, molecular evolutionary genetics analysis; MGE, mobile genetic element; MUSCLE, MUltiple Sequence Comparison by Log-Expectation; NCBI, National Center for Biotechnology Information; PATRIC, Pathosystems Resource Integration Center; RAST, Rapid Annotation using Subsystem Technology; SEED, System for Effective and Efficient Discovery; VF, virulence factor.

Fourteen supplementary figures and seven supplementary tables are available with the online version of the manuscript.

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Comparative and pan-genome analysis of the complete genomes of the periodontal pathogen *Tannerella forsythia* reveals unique tetracycline resistance protein Q and GT22 carbohydrate-active enzyme family in strain 92A2

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ABSTRACT

Tannerella forsythia is a key periodontal pathogen associated with chronic periodontitis, yet comprehensive genomic comparisons based on complete genomes remain limited. In this study, we conducted a comparative and pan-genome analysis of all available complete genomes of *T. forsythia* using various bioinformatics tools to identify core, accessory and unique genes and their functional annotations. Phylogenetic analyses based on all 59 and 27 high-quality genomes revealed intra-cluster divergence supported by ANI and dDDH comparisons. Comparative genomics revealed the existence of a conserved core genome with strain-specific variations linked to virulence and antimicrobial resistance, coupled with genome plasticity. Specifically, the tetracycline resistance gene *tet(Q)* was identified solely in *T. forsythia* 92A2 (found in 3 copies) and not in KS16 or 3313, suggesting possible strain-specific resistance. KEGG pathway analyses indicated that 74 core pathways were conserved across all strains, 13 were shared between 92A2 and 3313, 2 were shared by KS16 and 92A2, and KS16 and 3313 exclusively shared none. CAZyme analysis revealed conserved profiles with strain-specific differences and a unique GT22 family in strain 92A2. The pan-genome held 3575 gene clusters with 61.6 % core genes and 22.6 % strain-specific genes. These results provide the first complete genome-based pan-genomic profile of *T. forsythia*, indicating its genomic diversities, prospects of antibiotic resistance, and its conserved metabolic traits.

1. Introduction

Chronic infections that cause inflammation and tissue destruction around the teeth, which we know as periodontal diseases, are among the most common diseases worldwide and a significant public health concern [1]. These diseases involve a complex assemblage of microorganisms, and *Tannerella forsythia* (*T. forsythia)* is one of the most important pathogens in periodontal disease, particularly in its more advanced stages [2,3]. *T. forsythia*, first characterized and isolated in the 1970s, was initially described as a fusiform Bacteroides; it was later placed within a group with *Porphyromonas gingivalis* and *Treponema denticola,* which has become widely recognized as the "red complex" of bacteria and is very strongly associated with the more advanced forms of periodontal disease [4,5]. Despite the clinical relevance of *T. forsythia*, we know little about the genetic background of *T. forsythia*, and particularly, we do not know much about the basis for its virulence, adaptability and survival mechanisms applied in a complex oral environment [6,7].

Comparative genomics and pan-genomes represent compelling tools to explore the total gene content of a bacterial species [8]. Comparative genomics would also help determine the core genes found only in a specific bacterial species and differences between accessory genes unique to or present in variable numbers in different strains of the same bacteria [9]. Previous research has already confirmed possible virulence factors in *T. forsythia*, such as the characteristic glycoprotein S-layer, proteolytic enzymes, and biosynthetic pathways to produce host-interacting molecules [7,10,11]. However, it is not known if these features hold across strains from different geographic or clinical origins.

In this study, we performed a comprehensive comparative and pan-genome analysis of all available complete genome sequences of

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ORIGINAL PAPER

Comparative genomics reveals genetic diversity and differential metabolic potentials of the species of *Arachnia* and suggests reclassification of *Arachnia propionica* E10012 (=NBRC_14587) as novel species

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Abstract

The genus *Arachnia*, including *Arachnia propionica* and *Arachnia rubra*, are part of the normal oral and respiratory microbiota but can act as opportunistic pathogens in humans. This study investigates the functional, phylogenomic and taxonomic characteristics of 10 completely sequenced *Arachnia* strains, to elucidate their evolutionary relationships and divergence patterns, focusing on genomic variability and functional diversity. Phylogenetic analyses revealed distinct patterns, with *Arachnia propionica* strains showing significant divergence compared to the conserved *Arachnia rubra* strains. Notably, E10012 (=NBRC 14587) emerged as a distinct lineage with unique adaptations, while NCTC11666 exhibited a unique phylogenetic position, suggesting subspecies-level classification. Functional analyses highlighted variability among *Arachnia propionica* strains, with E10012 (=NBRC 14587) showing genes linked to choline metabolism and metal resistance, and NCTC11666 enriched in carbohydrate-active enzymes like GH179. In contrast, *Arachnia rubra* demonstrated genomic conservation, indicative of evolutionary specialization. This study reveals that strains E10012 (=NBRC 14587) and NCTC11666 displayed unique genomic features and distinct phylogenetic positioning, suggesting their reclassification as potential novel species and subspecies respectively. This underscores the balance between genomic conservation and diversification in *Arachnia*, reflecting their ecological adaptability and functional roles in the oral microbiome.

Keywords *Arachnia* · *Arachnia propionica* · *Arachnia rubra* · Phylogenomic analysis · Comparative genomics · Novel species

Abbreviations

ABC transporters	ATP-binding cassette transporters
Alr	Alanine racemase
AMR	Antimicrobial resistance
ANI	Average nucleotide identity
BGCs	Biosynthetic gene clusters
BLASTn	Basic Local Alignment Search Tool for nucleotides
BPGA	Bacterial pan genome analysis
CARD	Comprehensive Antibiotic Resistance Database
CAZYME	Carbohydrate active enzyme
CBM	Carbohydrate-binding module
CDs	Coding sequences
CE	Carbohydrate esterase
COG	Clusters of Orthologous Groups
Dbcan	Database of Carbohydrate-Active Enzymes
DDH	DNA–DNA hybridization
Ddl	D-Alanine-D-alanine ligase
DNA	Deoxyribonucleic acid
EF-G	Elongation factor G
EGGNOG	Evolutionary genealogy of genes: non-supervised orthologous groups

Communicated by Huayue Li.

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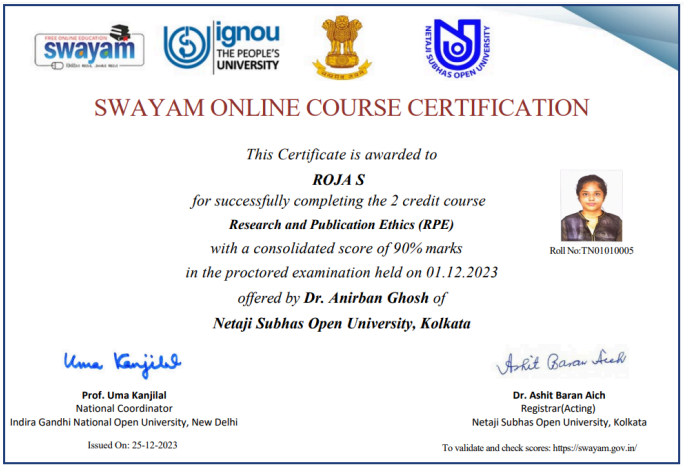

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International Conferences:

Presented the research work at international conferences (ICER-ID 2025 and PAGE-25) on oral microbiome resistome profiling and comparative genomics, gaining global scientific exposure.

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International Conference on Emerging and Re-emerging Infectious Diseases
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at the International Conference on Emerging and Re-emerging Infectious Diseases (iCER-ID 2025) held on 17th and 18th February 2025, at CSIR-SERC, Taramani, Chennai, India.

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Dr. J. Jeyashambavi, Organizing Secretary
Dr. S. Ravikumar, Organizing Chairperson
Dr. R. Jawahar, Organizing Chairperson
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10 CDE

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Ms. Roja S
has won the Best Oral/Best Poster Award at the 6th MICROSUMMIT-2025: International Conference on Cutting-Edge Strategies in Epidemiology and Diagnostics of Infectious Diseases (CESEDID-2025) held at Saveetha Dental College & Hospitals, Chennai, on the 1st and 2nd of August, 2025, organized by the Department of Microbiology in association with the Indian Association of Applied Microbiologists (IAAM), and co-sponsored by ANRF and CSIR, New Delhi.

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